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Some promising control measures for the management of *Meloidogyne* spp. on "Gotukola"

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Abstract

Cultivars of ***Centella asiatica*** were found to be infected by species of ***Melodogyne,*** a root knot nematode. Pot experiments under greenhouse were conducted to determine the effect of this nematode on the yield of these plants and attempts were also made to investigate suitable control measures. The use of resistant plants is a highly satisfactory method, which does not cause environmental pollution. In this study six cultivars of ***Centlla asiatica*** were tested for their susceptibility to this nematode. Both cultivars of high yielding type were found to be less resistant to ***Meloidogyne*** spp. whereas var. ***Mirigama*** was moderately resistant. Only one was found to be highly resistant among the types tested.

The loss of yield due to nematode infection was considerable in both cultivars of high yielding type compared to others, and a 29% loss in the yield was observed in the most susceptible culitvar tested.

Meloidogyne spp. were effectively controlled using compost. Application of soil: sand: compost in the proporation of 1:1:4 completely inhibited, and the same mixture used at 1:1:2 satisfactorily inhibited the nematode population giving maximum yield of ***Centella asiatica*** under the conditions tested.

Key words: *Centella asiatica, Meloidogyne,* root knot nematode

1. Introduction

"Gotukola" is a vernacular name of a group of plants, which belong to the family umbelliferae. *Centella asiatica* is one member, which is commonly known as "Hin-gotukola" (Dassanayaka and Fosberg, 1981). The plant has cultivars with different morphological characters (Perera, 1990 and Perera, 1996).

Centella asiatica is a rich source of iron, phosphorus, calcium and other minerals and also contains high percentage of vitamin B. Other than the nutritional value, it has medicinal importance too. For eg. *C. asiatica* has been reported to employed as diutetic in several diseases (Duster, 1977). Further, it is also rich in essential oils suggesting its importance in industry. The edible qualities, pleasing taste and medicinal properties have rendered its popularity as a green leafy vegetable in the diet of Sri Lankans. Because of the ease of cultivation, it has become popular among commercial growers in the recent past for both local and export purposes. However, the yield of "gotukola" is limited due to unruly attacks of diseases caused by pathogens of several groups. Among these, a nematode disease caused by species of *Meloidogyne* is disastrous. The damage caused by ***Meloidogyne*** spp. on *C. asiatica,* is becoming increasingly conspicusous in many districts of Sri Lanka.

In Sri Lanka, an association of ***Meloidogyne*** spp. with ***C. asiatica*** was first reported by Sivapalan in 1978 and then by Ekanayake and Toida in 1997. However, details of the disease such as its effect on yield, susceptible cultivars, control measures etc. have not been reported. A preliminary study made in 1996, revealed that the nematode caused gall formation in *C. asiatica* within 3 weeks of planting under greenhouse conditions and the infection could be successfully controlled by a systemic nematicide, carbofuran (Salim, unpublished results).

Since "gotukola" plants are consumed as raw salad / drink, chemical control cannot be recommended. Use of resistant varieties, field sanitation and good crop husbandry may help to keep down the attack of the disease.

Therefore, the present work was planned with the objective of identifying different cultivars/varieties of ***C. asiatica,*** screening of these cultivars for susceptibility of ***Meloidogyne*** spp. and determining the effects of ***Meloidogyne*** spp. on the yield of each cultivar identified. This was further extended to study the effect of compost content on the level of infection, which could be used as an inexpensive, non-chemical control measure that is environmentally acceptable.

2. Materials and methods

Culture of nematodes.

Soil samples were collected from a private garden at Narahenpita where *C. asiatica* plants (cultivar unknown) were growing. These infected plants were stunted with yellow leaves and galled roots. Both roots and soil were checked for the presence of ***Meloidogyne*** spp. Soil samples were brought to the greenhouse at the University of Sri Jayawardhenepura and the nematodes were cultured on the same cultivar of *C. asiatica* in clay pots. One part of steam sterilized soil, one part of steam sterilized sand, and one part of steam sterilized compost were mixed thoroughtly with one part of nematode infested soil and used as a potting mixture to culture the nematodes.

Maintenance of test plants.

Top soil was collected from the University Botanical garden and sieved to remove extraneous matter. A soil mixture was prepared using two parts of top soil, one part of sand and one part of compost. This mixture was thoroughly mixed and steam sterilized under 15 lb/inch^2 pressure at 121° C for 20 minutes.

Several cultivars of *C. asiatica* collected from different parts of the Island (Horana, Boralesgamuwa, Ratnepura, Narahenpita and Polonnaruwa) were propagated in 6 x 10 cm polythene bags with soil mixture prepared as above. Plants were watered daily and maintained in the greenhouse at 31±2° C. In addition to these, *C. asiatica* "Mirigama" variety was obtained from HORDI (Horticulttural Crop Research and Development Institute) at Gannoruwa and propagated under the same greehouse conditons.

Differentiation of cultivars of *C. asiatica* using morphological characters.

Plants were differentiated using morphological characters described by Perera (1990) and Perera (1996). An average of 25 samples were taken to express the data of each character studied.

After differentiation they were designated as G_1, G_2, G_3, G_4, G_5 and G_6. Identification of G_1 - G_6 was done using Triman's description given in Flora of Ceylon (part 3).

Investigation of susceptibility of G_1 - G_6 to *Meloidogyne* species

Soil medium was prepared as described above. Uniform sized cuttings with three leaves, leaf bud and some roots from each cultivar, were planted as two plants per pot in clay pots of 8cm diameter. Each treatment had five replicates. All pots were then arranged randomly in a greenhouse at 31±2° C. The plants were watered daily using an equal volume (50ml) of water which was applied to each pot twice a day. Then plants were uprooted and the level of infection was evaluated as mentioned below.

Effect of compost on the population of nematode

The cultivar, G6 was selected for this experiment as it was found to be the most susceptible line tested. The soil mixtures were prepared as follows:

T (Treatments)	Sterilized top soil: Sterilized sand: Compost/ml
T1	1:1:0
T2	1:1:1
T3	1:1:2
T4	1:1:4
T5	1:1:6
T6	1:1:8
T7	0:0:1

Equal amounts of nematode infested soil were separately added to all the above treatments and were mixed thoroughly. Two, uniform sized plants (with three leaves, leaf bud and some roots) were introduced to each treatment (10 cm diameter plastic pots) with five replicates.

Assessment of infection / disease

Plants were allowed to grow for 60 days after inoculation with nematodes. They were then uprooted carefully avoiding damage of roots and washed free of soil Thereafter, root system were observed for root knots which wre counted in order to assess the level of infection and final nematode population. Root systems of different cultivars were rated according to Tayler and Sasser (1978) galling index as given below.

0	no galls	-	Immune
1	1-2 galls	-	Resistant
2	3-10 galls	-	Moderately susceptible
3	11-30 galls	-	Moderately susceptible
4	31-100	-	Moderately susceptible
5	>100 galls	-	Highly susceptible

Fresh and dry weights of shoots and roots of each tratment were also determined for growth parameter. Dry wights of shoots and roots were taken in each treatment by drying the plant material in an oven at 105° C until a constant weight was obtained.

Experimental design and statistical analysis

A randomized block design was used to arrange the pots on the bench in the greenhouse. Data were subjected to analysis of variance (ANOVA) and t-test where appropriate, to test for interactions among and between treatments and in the absence of interactions, to test treatment effects. Significant differences between treatment means were determined by the ANOVA F test and t-test.

3. Results

Many morphological variations were observed among the types of cultivars collected. According to the characters considered, it was possible to differentiate these into six groups designated as G_1-G_6 (Figs. 1-6). Table 1 shows the morphological charcters of different cultivars of *C. asiatica.*

Table 1. Some morphological characters of differnt cultivars of *C. asiatica.*

	G_1	G_2	G_3	G_4	G_5	G_6
Petiole length (cm)	3.45 ±0.92 Short	8.50±1.53 Short	5.80±0.90 Short	14.86±1.35 Medium	19.29±1.75 Long	22.67±1.97 Long
Internode length (cm)	6.30±0.1.2 Short	7.70±0.71 Short	5.50±0.70 Short	10.00±1.21 Medium	14.00±1.51 Medium	15.96±1.22 Long
Leaf area (cm^2)	6.35±0.16 Small	9.22±1.25 Small	11.09±0.24 Small	8.14±0.63 Small	27.70±2.40 Large	30.50±1.51 Large
Texture of the leaf	Thin	Thin-medium	Thin	Thick	Medium	Thick
Colour of the leaf	Pale green	Pale green	Light green	Green	Dark green	Green
Margin of the leaf	Slightly dentate	Deeply dentate	Dentate and arranged in a pattern	Slightly entire	Dentate	Crenate

The data were based on the mean of 25 samples.

Criteria given for lengths,

<10cm - short, 10-15cm-Medium, > 15cm - Long

Criteria given for leaf area,

<15 cm^2 - Small, 15-20 cm^2 - Medium, > 20 cm^2 - Large

The screening trial conducted under greehouse conditions using above cultivars showed that they vary in their susceptibility to the nematode species. The mean number of root knots formed in each cultivar is shown in fig. 7. G_1 had few root knots whereas G_5 and G_6 had more root knots. When the results were analyzed using Tailor and Sasser galling Index, it was found that, G_1 was highly resistant, G_2, G_3 and G_4 were moderately susceptible whereas G_5 and G_6 were highly susceptible (Table 2).

Table 2. Galling index and degree of susceptibility of cultivars / varieties of *C. asiatica.*

Cultivar / variety	Galling index
G_1	1 HR
G_2	2 MS
G_3	3 MS
G_4 ***C. asiatica,*** var. 'Mirigama'	4 MS
G_5	5 HS
G_6	5 HS

HR- Highly resistant MS - Moderately susceptible HS -Highly susceptible

The effect of nematode infection on the yield (assessed as fresh weights of shoots) of each cultivar is shown in Fig. 8 The results indicate that there is no significant effect of nematode infection on the fresh and dry weights of shoots / yield of G1. But the infection caused signifiant effect on the yield of all the other types (G_2-G_6) tested (p=0.05).

Fig. 9 shows the percentage loss of yield of each cultivar. In the highly susceptible cultivars G_5 and G_6, the loss of yields were about 21% and 29% respectively. Among the moderatedly susceptible types, although

G_4 (Mirigama variety) had high galling index, lower percentage yield loss (9%) was observed when compared to G_2 and G_3 types where the loss of yields were found to be abut 12% and 15% respectively. The yield of G_1 was least affected by the infection and showed only about 4% loss compared to that of 'healthy' plants.

The results show that the infection has no significant effect on the fresh and dry weights of the roots of G_1, G_2 and G_3. However, a significant increase in the fresh and dry weights of roots was observed in G_4, G_5 and G_6 when compared to respective 'healthy' plants, probably due to the numerous galls in their root systems (Fig. 10).

The results also show that there exists a co-relation between compost content and the level of infection (Fig. 11) Root knots were not formed in plants grown in compost alone (T_7) and higher amounts of compost (T_4, T_5, T_6). Galls were formed only in those plants that were grown in the complete absence of compost, (T_1) and lower amounts of compost (T_2, T_3).

The fresh and dry wights of shoots collected from each treatment are shown in Table 3. The results showed that the fresh weights of shoots (leaf yield) in plants grown in the absence of compost, (T_1) were reduced when compared to other treatments. Thereafter, fresh weights of shoots were increased with the increase of compost content up to T_4 where maximum growth was obtained. Fresh weights of shoots were then reduced despite further increase of compost contents, although no infection was found in plants grown in these potting mixtures.

The fresh weights of shoots were minimum in plants grown in compost alone (T_7). Similar results were obtained for dry weights of shoots.

The mean fresh and dry weights of roots in each treatment is shown in Table 4. Accordingly the reduction of fresh weights of roots with the increase of compost was not significant. However, greater reduction of the weights of roots were obtained in the plants grown in compost alone (T_7). Similar results were obtained for dry weights of roots.

Table 3. Effect of compost content on the fresh and dry weights of shoots of *C. asiatica* (Cultivar G_6)

TREATMENTS	MEAN FRESH WEIGHTS OF SHOOTS (g)	MEAN DRY WEIGHTS OF SHOOTS (g)
T_1	6.8664 ± 0.139 (10)	0.8664 ± 0.013 (10)
T_2	8.6489 ± 0.259 (9)	0.7664 ± 0.023 (9)
T_3	8.9712 ± 0.165 (10)	1.0834 ± 0.016 (10)
T_4	9.1197 ± 0.170 (10)	1.1296 ± 0.017 (10)
T_5	8.5298 ± 0.164 (9)	1.1420 ± 0.012 (10)
T_6	8.2192 ± 0.174 (8)	1.0872 ± 0.020 (9)
T_7	5.8233 ± 0.092 (9)	0.6300 ± 0.011 (9)

The data were based on the average of plants given in brackets

Table 4 :- Mean fresh and dry weights of roots of *C. asiatica* (Cultivar G_6) treated with different compost contents .

TREATMENTS	MEAN FRESH WEIGHTS OF ROOTS (g)	MEAN DRY WEIGHTS OF ROOTS (g)
T_1	1.7706 ± 0.011 (10)	0.2270 ± 0.001 (10)
T_2	1.8337 ± 0.010 (9)	0.2303 ± 0.001 (9)
T_3	1.8444 ± 0.011 (10)	0.2295 ± 0.001 (10)
T_4	1.8433 ± 0.008 (10)	0.2335 ± 0.001 (10)
T_5	1.7958 ± 0.007 (9)	0.2256 ± 0.001 (9)
T_6	1.7167 ± 0.013 (8)	0.2073 ± 0.125 (8)
T_7	1.2668 ± 0.012 (9)	0.0842 ± 0.001 (9)

The data were based on the average of plants given in brackets

4. Discussion

Meloidogyne spp. were isolated from *C. asiatica.* The nematodes induced infection in all the cultivars of *C. asiatica* tested and produced characteristic root knots in each host plant. Presence of root knots is a clear indication of the disease incidence and the number of root knots is one of the parameters whch indicates the nematode population in the host and the degree of susceptibitliy of each host to the infection.

The present investigation showed that the degree of susceptibility of the plants to *Meloidogyne* infection varies with cultivars of *C. asiatica.*

Both high yielding cultivars tested were found to be the most susceptible hosts. Among the cultivars tested, a runner type which bears smaller leaf area (Fig. 1) was found to be the most resistant host from the types tested.

The most important plant species are highly to moderately susceptible where reproduction of the nematode takes place with large number of larvae entering the roots and producing many eggs. These plants are most likely to be damaged in the field, where the population increases rapidly. A small infestation in the soil at the beginning of the growing season can lead to a heavy infestation by the end of the season with a high reduction in the yield. The present investigation showed that even under greenhouse conditions the crop losses of cultivars of G_5 & G_6 were considerably high (21% and 29% repectively).

The compost content had a significant effect on the nematode population of soil. This investigation provides the first definitive information about the suitable compost content where maximum yield of *C. asiatica* can be obtained in additon to suppression of the level of infection. Complete reduction of the level of infection was found in plants that were grown in compost alone (T_7) and in the media to which 4, 6 and 8 parts of compost were added (treatments T_4, T_5 and T_6). The level of infetion was also satisfactorily reduced in the T_3 treatment where 2 parts of compost were added (Fig 11). Where the yield of the plant is concerned, the treatment T_4 was found to be the best, where maximum yield could be obtained, however, this was not significant (P=0.05) when compared with T_3 (Table 3).

Reduction of the yield was found with further increase of compost although the level of infection was reduced. In this instance although there was no nematode infection, unfavourable soil conditions due to limitation of other soil factors may have contributed to the adverse effects on the plant growth. Therefore, when considering the level of infection, yield of plant and the economic importance the application of a mixture of soil at the ratio of 1:1:2 can be recommended as a highly satisfactory method of controlling the infection under greenhouse conditions.

In biological control, parasites and predators of nematodes in the soil should increase in order to increase the mortality of plant nematodes. The additon of organic matter increases the population of certain fungi and predatory nematodes, which feed on plant parasiitic nematodes (Mehrotra, 1980). Alternatively, the organisms can be introduced directly to the soil. The parasitic organism, *Pasteuria penetrans* has been directly introduced to control *M. Javanica* in tomato, cow pea and navy bean by preventing reproduction of the female nematode (de Costa, 1992). However, in the present study, it was not possible to determine the role played by such organisms and further studies are needed.

In the recent past, the cultivar, G_6 has become very popular among the growers due to its fast growth and high yield compared to other types. High yieding crop varieties have proved to be highly susceptible to diseases which were preiously unimportant. These diseases have become widespread and causing damages quickly, where once these plants were grown a large scale. This was confirmed during the present investigation.

As the demand for these plants in the local and foreign market is high, a stisfactory control measure is the need of the hour. Chemical control cannot be recommended for a leafy vegetable like 'gotukola' Therefore, the present invesitgation provides promising resistant cultivars with a combination of compost as a successful and cheaper control measure for the management of *Meloidogyne* spp. on Gotukola which also prevents environmental pollution.

5. Acknowledgements

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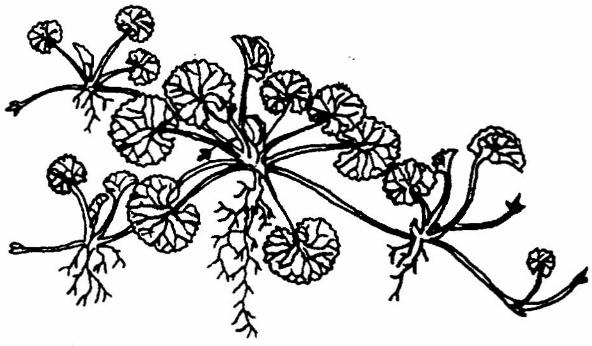


Fig 1:- Habit of *Centella asiatica* (G_1) (1x1/2)

Fig 2:- Habit of *Centella asiatica* (G_2) (1x1/2)

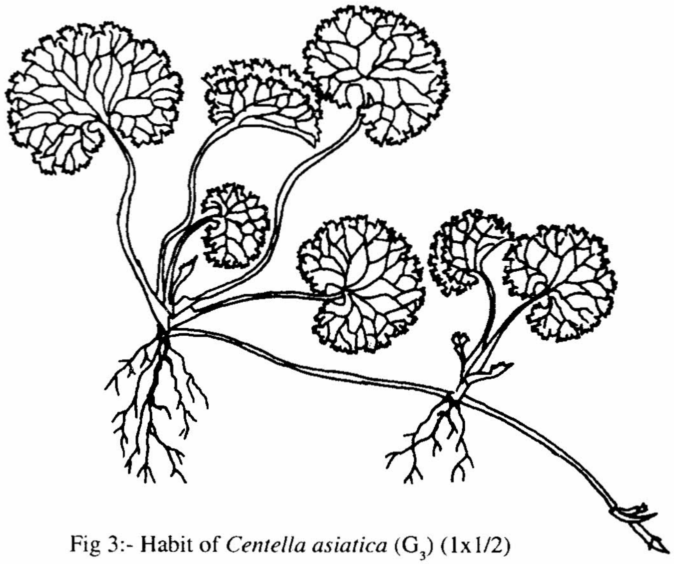


Fig 3:- Habit of *Centella asiatica* (G_3) (1x1/2)

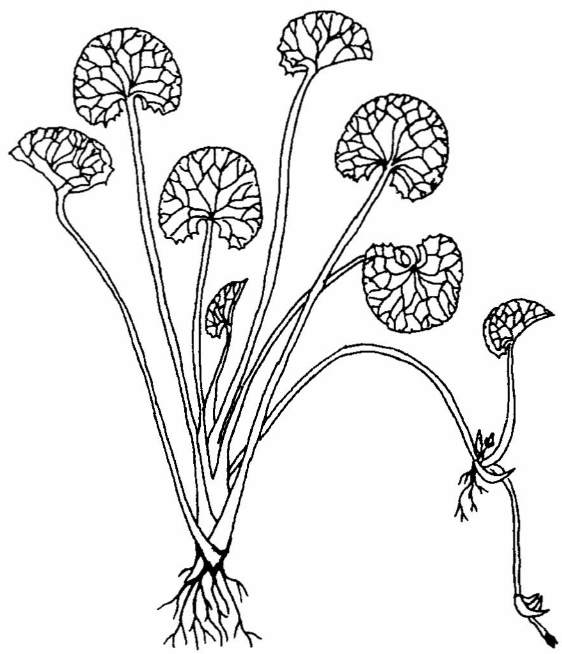


Fig 4:- Habit of *Centella asiatica* (G_4) (1x1/2)

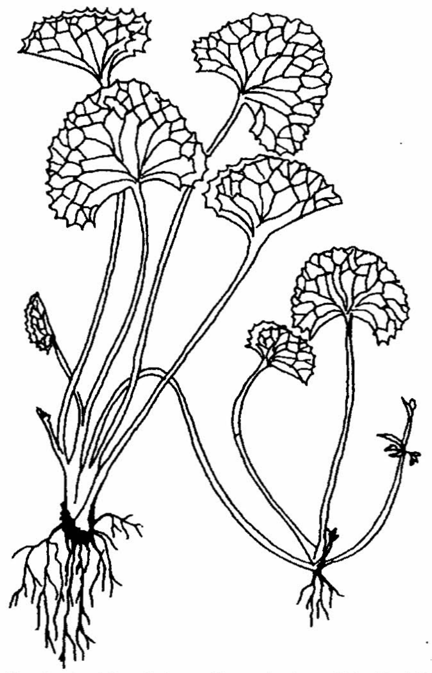


Fig 5:- Habit of *Centella asiatica* (G_5) (1x1/3)

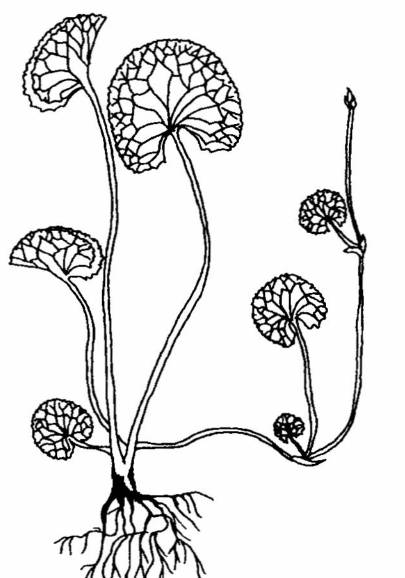


Fig 6:- Habit of *Centella asiatica* (G_6) (1x1/3)

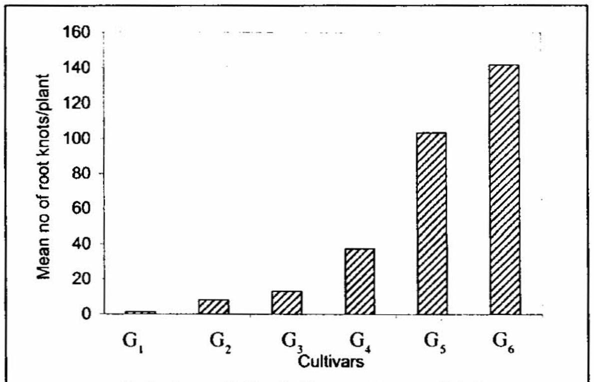


Fig 7:- Susceptibility of different cultivars to *Meloidogyne* spp.

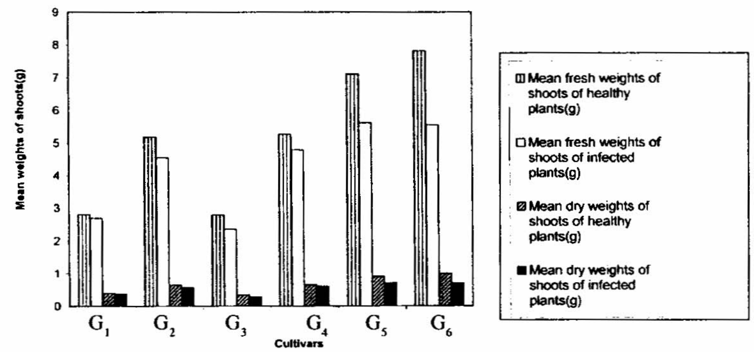


Fig 8:-Fresh and dry weights of shoots of healthy and infected plants of different cultivars

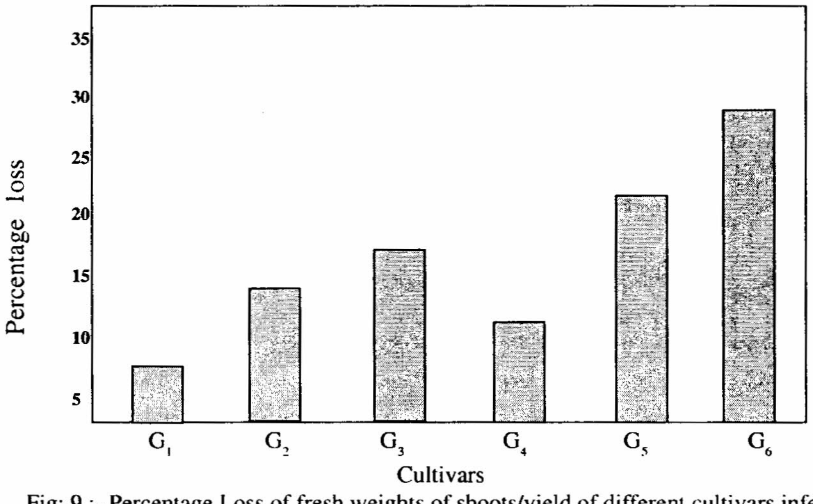
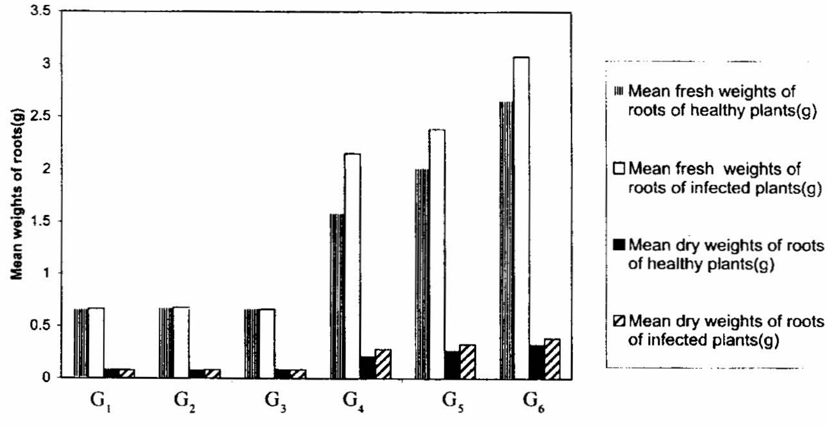
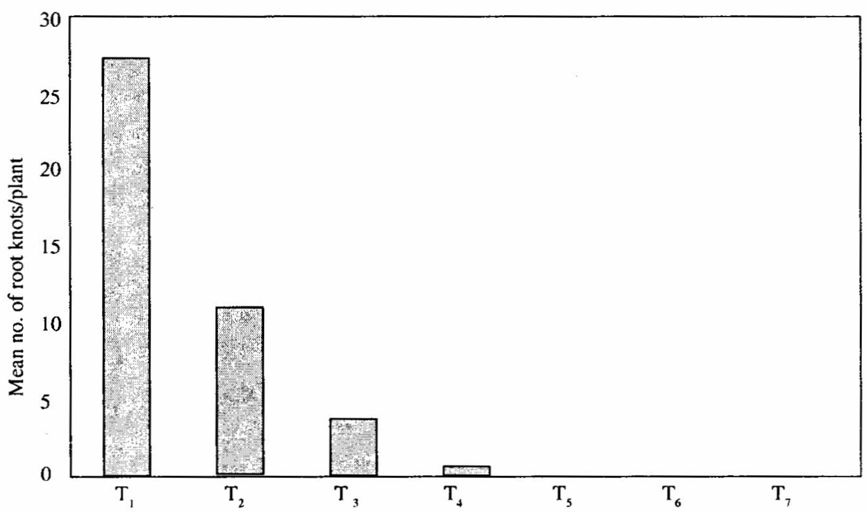


Fig: 9 :- Percentage Loss of fresh weights of shoots/yield of different cultivars infected with *Meloidogyne* species



Cultivars

Fig: 10 :- Fresh and dry weights of roots of healthy and nematode infected plants of different cultivars.



Treatments

Fig;- 11 Effect of compost content on the infection of *Meloidogyne* spp. in *C. asiatica* (cultivar G_6)